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16. ABSTRACT

A committee of scientists was established by the U.S. Environmental Protection Agency to appraise the current state of aneuploidy test methodology, to compile and analyze published data on the chemical induction of aneuploidy, and to provide guidance for additional test development and validation. The committee was organized into 9 working groups, representing different eukaryotic organisms ranging from fungi to whole mammals. The Environmental Mutagen, Carcinogen, and Teratogen Information Program at Oak Ridge National Laboratory identified and provided literature to the working groups. The 9 reports were prepared by the individual working groups. The reports include: (1) descriptions of the available testing approaches and general principles to be followed for protocol development, (2) criteria for evaluating data, and (3) recommendations for future test development and basic research. The evaluated results are presented in tables. For the positive and negative calls, the lowest effective dose tested or the highest ineffective dose tested, respectively, and the range of concentrations tested are provided. An overall summary report was prepared that summarizes the conclusions and recommendations of the 9 working groups and the chemical data base. It is hoped that these reports will provide a framework for further test development and validation of aneuploidy assays.

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The efficacy of Neurospora in detecting agents that cause aneuploidy *

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Summary

A total of 110 agents, 109 chemicals plus γ-rays, has been tested in a Neurospora pseudowild-type selection system for their ability to induce meiotic aneuploidy. 11 agents were positive, 47 were negative, and 52 were inconclusively tested. The system has a possible role as a short-term test for environmental agents causing human aneuploidy. The advantages of the system are its simplicity and ease of use. Disadvantages are its high variability of aneuploid frequency and an inability to distinguish mechanisms of aneuploidy.

* Report of the Aneuploidy Data Review Committee (Office of Research and Development, U.S. Environmental Protection Agency, Washington, DC).

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(1) Introduction

Neurospora crassa is one of the two best-studied filamentous fungi at the genetic level. The review of Perkins et al. (1982) shows the extensive number of marked loci and well-characterized chromosome rearrangements available for specific experimental purposes in this fungus. The availability of such special genetic stocks has made possible several approaches to the study of aneuploidy. Pioneering studies were done by Pittenger and his associates (e.g., Pittenger, 1954) who showed that pseudowild-type colonies (PWT) resulted from ascospores which were disomic for one chromosome ($n + 1$). These studies drew attention to the existence of aneuploidy in Neurospora and further showed that disomics are unstable and result in heterokaryons with two haploid nuclei representing each of the disomic chromosomes.

Threlkeld and Stoltz (1970) did tetrad analyses that pointed to two important mechanisms of meiotic aneuploidy: nondisjunction at either the first or second division and precocious centromere splitting at the first division.

Griffiths and Delange (1977) were the first to construct Neurospora strains specifically for the detection of aneuploidy. This system uses the frequency of PWT colonies as an indicator of aneuploidy production. These authors demonstrated that the system was sensitive to the induction of aneuploidy through the external application of chemicals by showing that *p*-fluorophenylalanine (pfp) increases the frequency of PWT colonies, and, by inference, the frequency of aneuploid (disomic) ascospores issuing from meiosis. This test system has been used to survey 110 agents (mostly chemicals) as possible inducers of aneuploidy in Neurospora. The results of these studies are contained in Griffiths (1979, 1980, 1981, 1982, unpublished data) and are summarized and reviewed in this paper.

A major reason for this extensive survey of agents as possible inducers of aneuploidy was the fear that there is an environmental component to human meiotic aneuploidy (Griffiths et al., 1979). It was argued that Neurospora is a reasonable choice for a short-term test system because, apart from the general convenience of a well-defined haploid organism, the chromosomes of Neurospora look and behave like typical eukaryotic chromosomes at meiosis (Barry, 1967). It should be emphasized as part of this point that Neurospora chromosomes are relatively simple to stain and study at the cytogenetic level, which distinguishes them from the chromosomes of yeast.

Although the Neurospora test system has been the most extensively applied, another system is available is a closely related organism, *Sordaria brevicollis*. Bond (1976) developed specially marked parental strains for the detection of aneuploid ascospores in situ in the ascus. These strains were heterozygous for two complementing recessive alleles that cause buff ascospore pigmentation. By surveying a large number of asci under the microscope, color patterns can be distinguished that reveal the occurrence of first- or second-division nondisjunction, precocious centromere division, and a curious phenomenon that can be explained only on the basis of an extra chromosome copy existing in the meiocyte (i.e., it was $2n + 1$). Bond and McMillan (1979) showed that this system is also sensitive to the external application of pfp and that it was the nondisjunction class that was increased and not the other two. Fulton and Bond (1983) reported more details of the system, but so far it has not been used in any routine survey of chemicals.

(2) Test description

(2.1.) Strains

The specially developed strains I-41-5 (used as conidial parent) and I-34-8 (used as protoperithecial parent) are diagrammed in Fig. 1. These strains are available for general use through the Fungal Genetics Stock Center, Aracata, CA (acquisition Nos. 3819 and 3820). The linkage relations shown in Fig. 1 are only approximate. The functions of the mutant alleles were as follows. The alleles *arg-1*, *ad-3A*, *ad-3B*, and *nic-2* are tight auxotrophic mutations which served as selective agents for the detection of prototrophic PWT colonies arising from aneuploidy (disomy) for chromosome 1. It has been demonstrated that the prototrophs are heterokaryons, bearing complementing nuclear types that reflect either parental or recombinant chromosomes 1 and that no $arg^+ad^+nic^+$ triple recombinants arise. The background frequency of

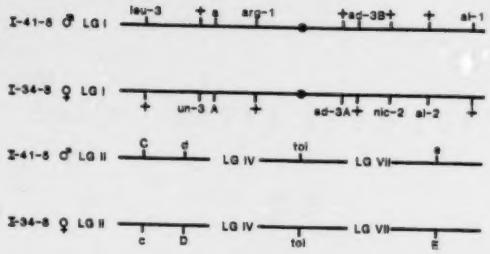

Fig. 1. Genotypes of parental strains used in the standard cross. I-41-5 and I-34-8 are the parental strain designations; LG, linkage group.

PWT colonies in the standard cross is low, suggesting a normal meiosis. The alleles *leu-3* and *un-3* are redundant in this study; leucine supplementation was routine but *un-3* requires no supplementation. The *tol* gene is a recessive suppressor of the mating-type incompatibility reaction and permits vigorous growth of the PWT heterokaryon. The complementing *al-1* and *al-2* alleles were originally extra chromosome markers but served no function in the present studies. The heterokaryon incompatibility genes *C/c*, *D/d*, and *E/e* served to prevent the formation of multiple disomics (D.A. Smith, 1975) to make the system more closely mimic human trisomic aneuploidy. These genes also served to contraselect the formation of heterokaryons by the chance fusion of the germ tubes of adjacent ascospores. As a further guard against scoring such fusions as aneuploids, the prototrophs were checked at 2 days after plating to make sure that growth emanated from one ascospore.

The two parental strains generally are highly fertile in crosses. Loss of fertility can occur after prolonged vegetative subculturing (a phenomenon often encountered in Neurospora), so strains are best kept on silica gel and a fresh culture made for each experiment. Fertility often can be restored by plating conidia and selecting a fertile derivative.

(2.2.) Protocol

Day 1. The crossing medium is prepared and dispensed into 140-mm diameter plates. The protoperithecial parent is inoculated. Incubation is at 25°C.

Day 8. Conidia of the now established protoperithecial parent are removed by suction in a vertical laminar-flow hood. A dense conidial suspension of the male parent is added and spread over the surface of the cross plate. Several hours (usually 6 h) later, the plates are exposed to the environmental agent. A wide range of doses is utilized in 5 or 6 crosses; the highest dose compatible with adequate fertility is eventually analyzed.

Day 30. Plating medium is prepared. Ascospores are harvested, suspended in 0.1% agar, heatshocked, and about 10^5 are plated on selective medium (usually on 20 plates). Simultaneously, a diluted suspension is plated (usually 5 plates) on supplemented medium to estimate total viable ascospores. Ascospore viability is usually > 90%.

Day 32. Young prototrophic colonies are examined to check for single ascospore origin.

Day 34/35. Colonies are counted and recorded. Aneuploid frequency is determined as the number of PWT colonies per total viable ascospores.

The above experiment constitutes one test. The cross showing the appropriate optimum dose may be repeated to provide several replicate values for each agent tested. (Experience with the system has shown that 5 values are probably a safe minimum.) With each treated cross, an untreated control cross is run concurrently and analyzed simultaneously. Often, one control cross served for several treated crosses run the same day with the same medium batch. Periodically, pfp is used as a positive control to ensure that the system is still sensitive.

The solvent used was water in the great majority of cases. Solutions of 4% dimethyl sulfoxide (DMSO) or 4% ethyl alcohol have been used for chemicals insoluble in water. At these concentrations, these latter two solvents have shown no appreciable effect to date on fertility or neuploid frequency.

Doses are reported in terms of the final concentrations in the crossing medium. The simple assumption is that dose at the target site will be in some way proportional to this concentration. In only a few experiments was a systematic attempt made to examine possible dose effects. In one such case, the addition of DMSO was shown to generate a dose effect for pfp (Griffiths, 1982). In general, doses affecting fertility were those analyzed, and often doses below the threshold

TABLE 1

SUMMARY OF AGENTS TESTED IN THE NEUROSPORA ANEUPLOIDY SYSTEM

Chemical	CAS Registry No.	Number of tests	Experimental mean ($\times 10^5$) [a]	Control mean ($\times 10^5$) [a]	Positive (+) negative (−), or inconclusive [b]	Dose range [c]	HIDT [d]	LEDT [e]	Fertility reduction [f]
1. 2-Acetylaminofluorene	53-96-3	6	13.3	14.5	−	500.0–200.0(3)	2000.0		yes
2. Acridine orange	65-61-2	6	14.7	14.7	−	100.0(1)	100.0		yes
3. Actidione	66-81-9	3	2.0	4.2	I [g]	0.009–0.01(2)			yes
4. Adriamycin	23214-92-8	11	11.1	27.8	I [h]	0.6–111.0(3)			no
5. Alloxan	50-71-5	9	8.1	7.6	−	100.0–2000.0(5)	2000.0		yes
6. Amethopterin	59-05-2	11	22.9	8.8	+	32.0–435.0(5)		32.0	no
7. Ametryne	834-12-8	6	7.5	7.3	−	10.0–100.0(2)	100.0		yes
8. *p*-Amino diphenylamine hydrochloride	2198-59-6	1	2.6	2.3	I [g]	8.3(1)			yes
9. 2-Aminofluorene	153-78-6	6	16.2	14.0	−	50.0–100.0(3)	100.0		yes
10. 2-Amino-4-nitrophenol	99-57-0	1	2.2	2.3	I [g]	8.3(1)			yes
11. 2-Amino-5-nitrophenol	121-88-0	1	1.5	2.3	I [g,h]	8.3(1)			no
12. *m*-Aminophenol	591-27-5	3	34.5	3.0	+	83.0–250.0(3)		220.0	yes
13. *p*-Aminophenol	123-30-8	1	0.5	3.1	I [g,h]	83.0(1)			no
14. Aminopterin	54-62-6	5	10.1	2.7	−	125.0–250.0(2)	250.0		yes
15. 3-Amino-1,2,4-triazole	61-82-5	5	9.3	8.9	−	100.0–500.0(3)	500.0		yes
16. Amphotericin B	1397-89-3	10	26.3	18.7	−	0.5–10.0(3)	10.0		yes
17. Atrazine	1912-24-9	19	10.3	2.7	+	10.0–500.0(5)		10.0	no
18. 8-Azaguanine	134-58-7	1	1.5	4.1	I [g]	10.0(1)			yes
19. Azure A	531-53-3	8	27.0	21.6	−	50.0–250.0(4)	250.0		yes
20. Azure B	531-55-5	8	18.3	13.8	−	1.0–200.0(5)	200.0		yes
21. Azure C	531-57-7	5	13.6	22.0	−	100.0–1000.0(2)	1000.0		yes
22. Benomyl	17804-35-2	5	22.0	17.4	−	0.1–1.0(2)	1.0		yes
23. Benzo[*a*]pyrene	50-32-8	5	11.6	11.2	−	100.0–1000.0(2)	1000.0		yes
24. Brilliant blue	2650-18-2	2	10.0	15.0	I [g]	100.0(1)			yes
25. Bromacil	314-40-9	6	7.6	2.6	+	10.0–100.0(2)		10.0	no
26. Caffeine	58-08-2	7	24.8	7.3	+	10.0–750.0(2)		750.0	yes
27. Chloral hydrate	302-17-0	1	4.2	6.5	I [g]	500.0(1)			yes
28. 4-Chlororesorcinol	95-88-5	3	4.5	2.6	I [g]	8.3–65.0(3)			yes
29. Cobalt chloride	7646-79-9	2	4.5	4.5	I [g,h]	10.0(1)			no
30. Colchicine	64-86-8	1	4.5	3.1	I [g]	500.0(1)			yes
31. *m*-Cresol	108-39-4	2	11.8	7.3	I [g,h]	0.01–0.1(2)			– [i]
32. Croton oil	8001-28-3	5	8.8	9.2	I [g,h]	1000.0–1500.0(2)			–
33. Cyanazine	21725-46-2	12	6.5	6.5	I [h]	10.0–500.0(4)			no
34. Cyclic AMP	60-92-4	8	6.9	7.7	−	100.0–200.0(2)	200.0	yes	
35. Cytochalasin B	14930-96-2	1	1.3	3.5	I [g,h]	42.0(1)			no
36. Cytoxan		5	10.8	9.4	−	150.0–500.0(3)	500.0		yes

37. 2,4-Diaminoanisole sulfate	39156-41-7	6	21.3	7.1	+	83.0–150.0(2)		150.0	yes
38. 2,5-Diaminoanisole sulfate	66671-82-7	13	7.3	6.2	I [h]	8.3–50.0(2)			no
39. 2,4-Diaminotoluene	95-80-7	5	12.2	8.9	–	20.0–63.0(2)	63.0		yes
40. Diazinon	333-41-5	6	9.3	10.7	–	0.0025%(1)	0.0025%		yes
41. Dichloran	99-30-9	7	14.1	15.1	–	5.0–10.0(2)	10.0		yes
42. Diethylstilbestrol	56-53-1	14	22.4	28.4	–	1.0–10.0(2)	10.0		yes
43. Dimethyl sulfoxide	67-68-5	3	9.3	8.5	I [g,h]	4 %(1)			no
44. Enterovioform	130-26-7	3	5.8	6.8	I [g]	25.0–50.0(2)			yes
45. Eptan	759-94-4	5	6.1	2.6	–	1.0–10.4(2)	10.4		yes
46. Ethanol	64-17-5	5	8.9	6.1	–	4 %(1)	4 %		yes
47. DL-Ethionine	67-21-0	5	7.9	6.3	–	1.0–2.5(2)	2.5		yes
48. Ethyl methanesulfonate	62-50-0	1	2.1	1.9	I [g,h]	100.0(1)			no
49. Ethylene glycol	107-21-1	6	10.0	7.5	–	0.03–0.06(2)	0.06		yes
50. Ferric chloride	7705-08-0	1	2.4	0.9	I [g,h]	91.0(1)			no
51. Folic acid	59-30-0	1	7.9	4.1	I [g,h]	–			–
52. β-Fluoropyruvic acid	443-48-7	4	6.0	4.8	I [g]	10.0–12.5(2)			yes
53. *p*-Fluorophenylalanine	60-17-3	42	24.5	4.3	+	0.5–10.0(9)		1.0	no
54. 4-Fluorotryptophan	25631-17-8	7	7.9	6.4	–	0.5–1.0(2)	1.0		yes
55. *m*-Fluoro-DL-tyrosine	403-90-7	4	19.8	26.0	I [g]	10.0–100.0(2)			yes
56. 5-Fluorouracil	51-21-8	5	3.4	4.9	I [h]	1.0–2.5(3)			no
57. 5-Fluorouracil deoxyriboside	50-91-9	6	10.4	7.1	–	10.0(1)	10.0		yes
58. Formaldehyde	50-00-0	9	13.6	11.8	–	0.01–0.5%(3)	0.5%		yes
59. γ-Radiation (40 r/sec)	–	18	14.3	5.6	+	5.0–15.0(2)		5.0	no
60. Haloprogin	777-11-7	5	8.7	6.5	–	0.01–10.0(2)	10.0		yes
61. Hycanthone	3105-97-3	2	1.8	3.1	I [g,h]	0.01–0.8(2)			no
62. 8-Hydroxyquinoline	148-24-3	4	16.3	5.2	I [g]	50.0–100.0(2)			yes
63. Isopropanol	67-63-0	4	9.0	6.3	I [g]	5.0–10.0%(3)			yes
64. Lucanthone	479-50-5	2	1.7	3.1	I [h]	0.1–0.4(2)			no
65. Malathion	121-75-5	5	16.4	13.4	I [h]	0.005–0.03%(4)			–
66. Maleic hydrazide	123-33-1	12	10.4	7.9	–	32.0–500.0(4)	500.0		yes
67. Mercuric chloride	7487-94-7	3	11.4	2.6	I [g]	0.009–0.1(3)			yes
68. Methanol	67-56-1	4	13.5	11.6	I [g]	10.0%(1)			yes
69. 8-Methoxypsoralen	298-81-7	4	16.0	13.0	I [g]	10.0–100.0(2)	100.0		yes
70. *N*-Methyl-*p*-aminophenol sulfate	55-55-0	1	0.4	1.7	I [g]	8.3(1)			no
71. Metronidazole	443-48-1	17	9.9	16.3	– [j]	500.0–2000.0(4)	200.0		yes
72. Mitomycin C	50-07-7	4	5.2	6.6	I [g]	2.0–8.7(3)			yes
73. 1-Naphthol	90-15-3	6	6.5	8.9	–	0.8–2.5(2)	2.5		yes

TABLE 1 (continued)

Chemical	CAS Registry No.	Number of tests	Experimental mean ($\times 10^5$) [a]	Control mean ($\times 10^5$) [a]	Positive (+) negative (−), or inconclusive [b]	Dose range [c]	HIDT [d]	LEDT [e]	Fertility reduction [f]
74. Neutral red	553-24-2	10	15.0	13.0	−	100.0–500.0(4)	500.0		yes
75. Nicotine	54-11-5	1	3.0	3.1	I [g]	2000.0(1)			yes
76. Nitrilotriacetic acid	139-13-9	5	7.6	8.8	−	10.0–1000.0(4)	1000.0		yes
77. *p*-Nitrobiphenyl	92-93-3	4	7.5	5.2	I [g]	55.0–77.0(3)			yes
78. 2-Nitrofluorene	607-57-8	3	6.6	5.8	I [g,h]	1.0(1)			no
79. 4-Nitro-*o*-phenylenediamine	99-56-9	4	4.2	5.2	I [g]	10.0–50.0(3)			yes
80. 2-Nitro-*p*-phenylenediamine	5307-14-2	1	2.3	1.7	I [g,h]	8.3(1)			no
81. 4-Nitroquinoline	3741-15-9	5	5.5	2.9	−	1.0–2.5(3)	2.5		yes
82. 4-Nitroquinoline-1-oxide	56-57-5	8	13.3	8.3	−	0.5–10.0(6)	10.0		yes
83. Nitrosoguanidine	70-25-7	2	3.1	2.9	I [g]	1.0–10.0(20			yes
84. Nystatin	1400-61-9	7	9.0	6.1	−	0.1–1.3(2)	1.3		yes
85. Orthene	30560-19-1	8	6.2	6.0	−	5.0–500.0(3)	500.0		yes
86. *o*-Phenylenediamine	95-54-5	2	4.0	1.7	I [g,h]	8.3–50.0(2)			no
87. *p*-Phenylenediamine	106-50-3	1	3.2	1.7	I [g,h]	8.3(1)			no
88. *m*-Phenylenediamine	108-45-2	1	1.2	2.2	I [g,h]	0.8(1)			no
89. Phenylmercuric nitrate	55-68-5	7	22.0	33.0	−	0.05–0.1(3)	0.1		yes
90. Potassium fluoride	7789-23-3	4	10.7	4.8	I [g]	62.5–100.0(2)			yes
91. Quercitin	117-39-5	5	10.6	4.2	−	100.0–1000.0(2)	1000.0		yes
92. Resorcinol	108-46-3	11	7.5	6.2	−	10.0–110.0(3)	110.0		yes
93. Saccharin	81-07-2	5	8.5	10.4	−	2000.0–14000.0(3)	14000.0		yes
94. Sarcosine	107-97-1	5	8.4	12.1	−	100.0–500.0(3)	500.0		yes
95. Shikimic acid	138-59-0	13	14.6	24.3	I [h]	1000.0–2400.0(3)			no

96. Simazine	122-34-9	12	5.7	6.5	–	10.0–500.0(4)	500.0		yes
97. Sodium fluoride	7681-49-4	3	9.9	4.6	I [g]	62.5–100.0(3)			yes
98. Stannous fluoride	7783-47-3	1	5.3	6.3	I [g]	100.0(1)			yes
99. Stilphostrol	522-40-7	12	7.2	2.8	–	10.9–1500.0(5)	1500.0		yes
100. Sulfacetamide	144-80-9	5	25.7	5.6	+	250.0–300.0(2)		250.0	yes
101. Sulfanilamide	63-74-1	4	7.8	2.3	I [g]	63.0–125.0(3)			yes
102. Terbacil	5902-51-2	6	11.0	7.3	–	10.0–100.0(2)	100.0		yes
103. Tolnaftate	2398-96-1	3	15.1	14.1	I [g]	1000.0(1)			yes
104. *p*-Toluene diaminesulfate	6369-59-1	2	3.2	3.6	I [g]	8.3–200.0(2)			yes
105. *o*-Toluidine	95-53-4	16	23.5	13.7	–(?) [k]	10.0–75.0(7)	75.0		yes
106. Trifluralin	1582-09-8	74	19.6	6.5	+	10.4–122.0(10)		10.4	yes
107. Trimethoprim	738-70-5	47	9.5	5.2	+	62.0–1500.0(7)		62.0	no
108. Urethane	51-79-6	8	20.9	24.0	–	1.0–100.0(5)	100.0		yes
109. Vinblastine sulfate	143-67-9	5	8.3	7.2	–	109.0–217.0(2)	217.0		yes
110. Zinc chloride	7646-85-7	2	5.0	1.9	I [g]	91.0–220.0(2)			yes

[a] Pseudowild-type colonies/10^5 ascospores.
[b] Based on ANOVAs, using 5% probability level.
[c] µg/ml unless written as a percentage; concentrations on the cross-plates. Number of dose points shown in parentheses.
[d] Highest ineffective dose tested.
[e] Lowest effective dose tested.
[f] Was fertility reduced in a reasonable proportion of tests?
[g] Inconclusive because of insufficient number of tests.
[h] Inconclusive because of inadequate challenge to the system.
[i] A dash indicates information not available.
[j] Produces a high proportion of white ascospores together with normal ones.
[k] Statistical results unexpected.

level were tried too. When a decrease of fertility could not be obtained easily, a test was often done anyway.

(2.3) Statistical testing

Exclusive use was made of one-way analyses of variance (ANOVA), and a separate calculation was made for each agent as the test was done. The 5% probability level was used routinely to decide on positive agents. One obvious problem here is that with 110 ANOVAs, the possibility of type I or type II statistical errors looms large (Sokal and Rohlf, 1973).

(3) Data presentation

(3.1) Collection of data

Since very few papers have been published on induced aneuploidy in Neurospora and since some data have not been published previously (Griffiths, 1980, unpublished), it was decided to be nonexclusive in the assembly of material for the present review. The summary to be presented is taken from the complete data sheets for all the agents tested; these sheets are available from the senior author and are also on file with the Environmental Mutagen Information Center (EMIC), Oak Ridge National Laboratory, Oak Ridge, TN 37831.

(3.2) Data summary

All the available data are summarized in Table 1. The various agents (mainly chemicals) are listed alphabetically together with their CAS Registry Number and the number of tests performed on each agent. In general, a minimum of 5 independent tests was to be considered a fair trial, but agents with fewer tests are included for the sake of completeness. It should be stressed that each independent test involves a separate cross. The untreated controls were run concurrently with the treated crosses. Usually, a batch of treated crosses was accompanied by one control cross. In the calculation of the control mean, a control value was included for each treated cross to provide proper weighting for the mean. However, for the analysis of variance calculations, only the unique control values were used to better estimate control variance. For example, if control values were 2.5, 2.5, 2.5, 3.0, 3.0, 1.8, and 1.8, the mean was calculated from all 6 values, but the ANOVA was calculated from only 3 entries: 2.5, 3.0, and 1.8. Both experimental and control mean units are PWT colonies/10^5 ascospores.

The positive and negative calls in column 6 are based solely on the results of probability estimates calculated from F values. In all cases the 5% level was used. Some agents that gave negative ANOVA results were reclassified as inconclusive, based on an inadequate number of tests (< 5) or an inadequate challenge to the system (no reduction of fertility).

The dose ranges are based only on tests in which a PWT frequency calculation was made. Usually a larger range of treatments was attempted: doses lower than those reducing fertility usually were not analyzed fully; doses so high as to severely limit fertility could not be analyzed because of insufficient ascospores. The highest ineffective dose tested (HIDT) and the lowest effective dose tested (LEDT) are shown in the eighth and ninth columns of Table 1 for positive and negative agents. The last column shows if a reduction of fertility was encountered in a reasonable number of tests. A typical 'yes' situation is one where the top 3 or 4 doses would show reduced fertility but the lowest dose would show normal fertility.

(4) Data interpretation

Of the 110 agents (109 chemicals plus γ-radiation), 11 gave a positive effect as defined above on the basis of statistical tests. All of the positive agents except one (*m*-aminophenol) were tested in excess of the 5 tests considered to be the minimum required as indicated by experience. A negative effect was found for 47 chemicals, and 52 were inconclusive. Thus, about 20% of agents fairly tested appear positive in their effects on PWT frequency and (by implication) aneuploid frequency.

The chemicals were selected on the basis of several criteria. There are those with a proven or suspected effect on chromosome misdivision (e.g., colchicine, benomyl, pfp). Second, there are agents of proven mutagenicity or carcinogenicity not previously tested for their effects on chromosome misdivision (e.g., acridine orange, benzopyrene,

ethyl methanesulfonate, nitrosoguanidine (No. 83)). Third, there are agents prevalent in the human environment. Overall, the list cannot be considered a random selection of chemicals; there was a marked enrichment for agents with demonstrated or suspected genotoxic or clastogenic effects.

The great majority of control values fall below $10/10^5$ ascospores. From experience in using the system, values above 10 are immediately suspect, either as a positive effect of an agent or as an unexplained deviation in a control experiment.

How much confidence can be placed in the positive or negative scores? This confidence must extend beyond simple acceptance of the statistical probability estimates. First, the positives fall into several groups. The amino acid analogue, pfp, is in a class by itself in consistently producing an elevated value. In fact it has been used as a positive control, and rarely disappoints in this regard. It is worth mentioning that the action of pfp on PWT frequency in Neurospora has been confirmed in preliminary results using a different selective system (B.R. Smith and Yorston, 1981), and it has also been shown to increase the frequency of presumed aneuploid-containing asci in Sordaria (Bond and McMillan, 1979). In agents such as pfp, which have been tested many times, confidence in its positive status is high. Indeed, a dose effect has been obtained for pfp in the presence of DMSO (Griffiths, 1982). Confidence in the remaining positive scores is also quite high; the smallest increase found is a doubling, with 3- and 5-fold increases being most common.

The negative scores probably contain some 'false' negatives. This is based partly on experience with the system (e.g., the statistical analysis of the *o*-toluidine data contradicts the feeling developed from extensive platings involving this agent. This explains the question mark alongside that negative score.). It is worth noting that of the 99 negatives and inconclusives, in 60 cases the treatment mean exceeds the control mean, and in 31 cases the control mean exceeds the treatment mean (the remainder show equality). This could indicate general weak effects of a large array of agents. Inadequate penetration by the test agent could contribute to false negatives.

The main problem with interpreting the data is the high variance encountered in these experiments. This is most pronounced in the case of the positive agent trifluralin, which occasionally shows values in excess of 100. The causes of the variance are not known. One possibility is variable challenge to the meiotic apparatus. Although the treatment procedures and culturing techniques were standardized as far as practically possible, it is a relatively common experience in culturing and crossing Neurospora (and other microbes) that uncontrollable variation is encountered. Features such as morphological abnormalities, difficultly in penetration of the mycelium by the test solution, reduced or small protoperithecia, and unusual ascospore germination are typical of such variation, and any of these features could result in variable PWT values. Another possibility concerns the tendency of ascospores to sediment rapidly; this is relevant in dilution, plating, and hemocytometer counting. (A hemocytometer is used to estimate ascospore densities prior to plating.) Suspensions are routinely handled in 0.1% agar of a syrupy consistency in an attempt to reduce such sedimentation. One final point here is that although the maximum test chemical concentration compatible with cross-fertility is used, the loss of fertility might result not from meiotic disruption but from male conidial lethality. This would result in an inadequate challenge.

Also of possible relevance is a Grigg effect (Grigg, 1958) in which prototrophic cells might fail to produce a colony in the presence of densely plated auxotrophic cells. An attempt was made to compensate for this effect by standardizing plating densities within what seemed to be reasonable limits ($10^5 \pm 10\%$). No consistent correlation has been detected between plating densities and PWT frequency (Griffiths and Delange, 1977).

(5) Conclusions

Neurospora has provided a relatively straightforward test system for the detection of meiotic aneuploidy. It is a rapid test system which can process many chemicals inexpensively. In practice, considerable skill and judgment are needed in the determination of correct exposures and in the manipulation of ascospore suspensions, but these considerations probably apply to most fungal

aneuploidy systems. Furthermore, the test system has been shown to be sensitive to externally applied agents.

The major theoretical disadvantage of the test is that the mechanism of aneuploidy is not determined. This is a drawback in terms of biological investigation, but not necessarily in terms of environmental mutagenesis, since the fungal end point and the human end point are directly parallel. In an ideal approach, a rapid prototroph selection method, such as that described here, would represent a first-level screen to be supplemented by a second-level test, possibly the buff ascospore system of Sordaria, which would determine mechanisms.

At the beginning of the present study, it was expected that agents with specific meiotic activity (referred to below as type I) would be rare. These agents would interact specifically with some component of meiosis and would either abort meiosis, contributing to sterility, or produce a high level of abnormal meiotic products comparable with that produced by meiotic mutants (Delange and Griffiths, 1980). In Neurospora, the most prominent manifestation of such irregularities would be a high proportion of aborted (white) ascospores. No agents of this potency were encountered. They could be sought effectively by looking for ascospore abortion, although care would be necessary to eliminate the possibility of rearrangements which can also produce abortion. Agents of this type should work effectively at low doses, and would be the type most hazardous to human populations. (Note that although metronidazole regularly produced a high proportion of white ascospores, the explanation is not in aneuploidy but in a specific interaction of this chemical with one undetermined allele on chromosome 1 (Griffiths, unpublished).

At another level, agents which interact with a wide spectrum of cell constituents (type II agents) were expected to be less rare. Presumably pfp is of this type. This amino analogue presumably owes its meiotic effect to the consequences of its general incorporation into protein. It is an efficient toxin in somatic cells of Neurospora, in addition to its meiotic effect, and these two actions may be due to its involvement in different cell processes.

It is probably safe to say that all the positive agents act as type II in Neurospora even though some (e.g., trifluralin) might be expected to act as type I. Several agents that were expected to act as type I were negative in the Neurospora tests (e.g., benomyl, colchicine, vinblastine). This lack of type I agents, even in an enriched sample of agents, could point to some unknown atypical aspect of Neurospora meiosis or to a meiotic insularity that cannot be penetrated even at the HIDT. It will be interesting to compare the effectiveness of a range of presumed type I agents in other fungal meiotic systems in carefully established parallel tests.

Finally, it should be noted that the most desirable short-term test is that which most effectively mimics the human meiotic system and not necessarily that which produces the highest proportion of positive agents. The present Neurospora system, or a modification of it, may yet prove to be such a test.

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